ULTRASTRUCTURAL CHANGES IN THE INFECTIVE LARVAE OF *NIPPOSTRONGYLUS BRASILIENSIS* IN THE SKIN OF IMMUNE MICE

by D. L. Lee

ABSTRACT

Infective stage larvae of *Nippostrongylus brasiliensis* are immobilized within two to three hours after penetrating the skin of mice that are immune to this nematode. The larvae become surrounded by host defense cells and bundles of collagen fibers. The cuticle is the first structure of the larva to be attacked; host defense cells may secrete a collagenase which attacks the cuticle. Disorganization of the hypodermis and underlying muscle cells follows destruction of the larval cuticle.

INTRODUCTION

Infective third-stage larvae of *Nippostrongylus brasiliensis* quickly enter the skin of rats and mice, and move freely through the dermis. Larvae in the skin appear to be more permeable to fixative than those on the surface of the skin; this difference may be related to the change in environment and temperature when the nematode enters the skin of the mammalian host. In primary infections, host cells seem not to attack the penetrating larvae (Lee, 1972a). Taliaferro and Sarles (1937) have shown that larvae which penetrate the skin of immune hosts become coiled and immobilized in the skin or lungs, disintegrate and, eventually, are phagocytosed. This paper describes the ultrastructure of infective larvae in the skin of immunized mice.

MATERIALS AND METHODS

Four mice, six weeks of age, were each exposed to 500 infective-stage larvae of *Nippostrongylus brasiliensis*. Hair was clipped from an area on the left side of the abdomen of each mouse, and the larvae were applied to this area in a drop of water. The mice were physically restrained until the water had evaporated (five minutes). The initial exposure to 500 larvae was repeated two and

D. L. Lee is Professor of Agricultural Zoology at the University of Leeds.

four weeks later. Six weeks after the initial exposure to the larvae each mouse was exposed to 1000 larvae. These larvae were applied as before, but to an area on the right side of the abdomen. The larvae were allowed to penetrate for two or three hours. After the mice had been killed, the area of skin containing larvae on the right side of the abdomen was removed, chopped into pieces in cold 2.5% gultaraldehyde in cacodylate buffer (pH 7.0), and fixed at 4° C for 24 hours, or chopped into pieces in 1% osmium tetroxide (Rosenbluth, 1965) and fixed for 2 hours at 4° C. The fixed tissue was washed in buffer, dehydrated in ethanol, transferred to propylene oxide and then embedded in Araldite. Sections were cut on a Huxley ultramicrotome or an LKB Ultrotome III, mounted on formvar-coated grids, stained in a 5% solution of uranyl acetate in methanol followed by lead citrate, and viewed by means of an AEI6B or a Philips EM300 electron microscope.

RESULTS

Larvae were present in the dermis two to three hours after application to the skin, and most of them were coiled. Initial observations suggested that the worms were immobilized but were otherwise normal, as normal structural features such as esophagus, intestine, and muscles were readily apparent. Closer investigation, however, revealed a number of significant alterations in

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ABBREVIATIONS FOR FIGURES

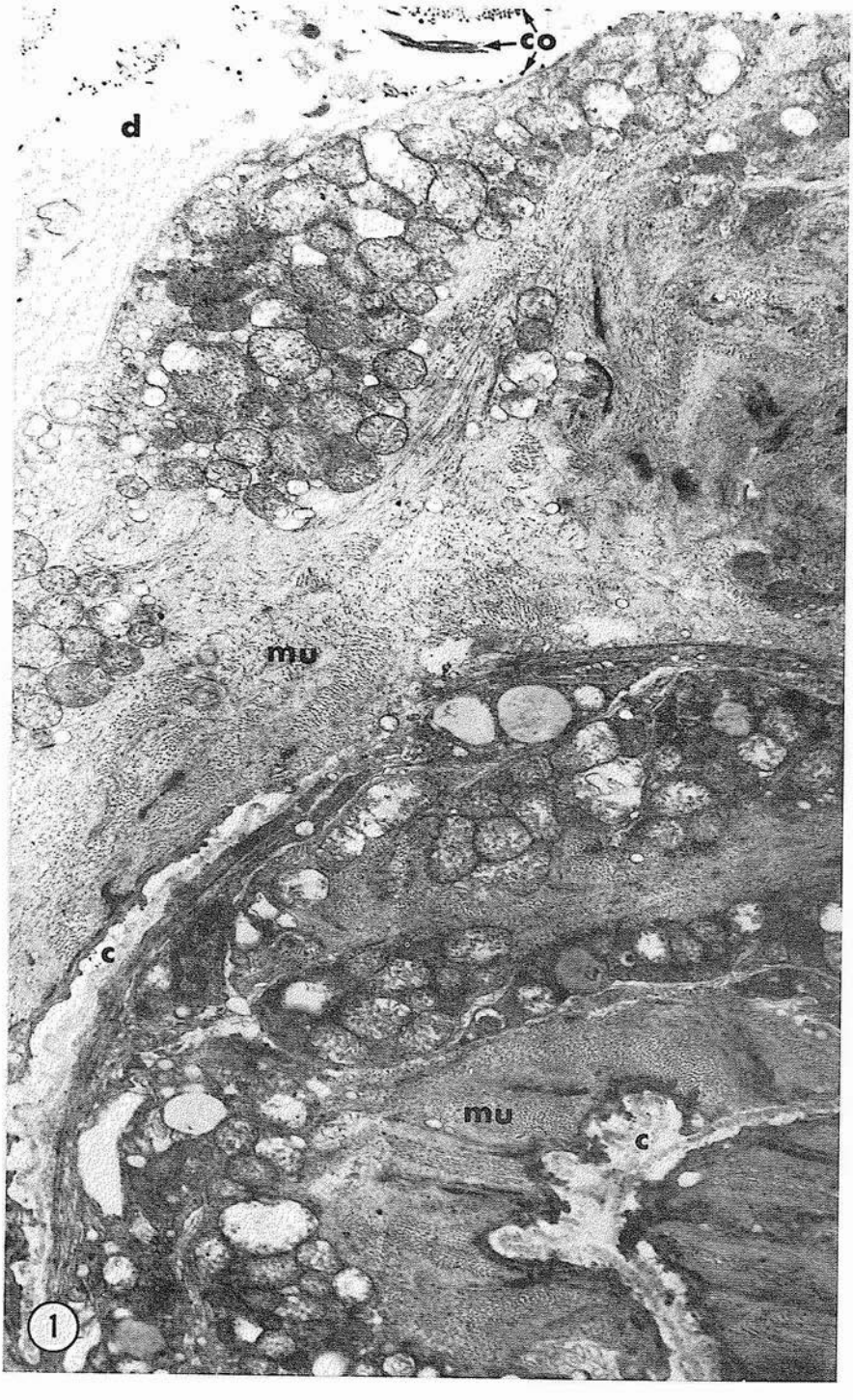
c, degenerating cuticle of body wall; *co*, collagen fibers of host origin; *d*, dermis of host skin; *h*, hypodermis; *m*, mitochondria; *mu*, muscle of body wall; *my*, myofilaments; *o*, esophagus; *oc*, esophageal cuticle.

FIG. 1. ELECTRON MICROGRAPH OF A SECTION THROUGH A COILED UP, INFECTIVE-STAGE LARVA of *N. brasiliensis* in the skin of an immune mouse. The larva is sectioned in three places with the outermost part of the coil in the top of the picture and the parts of the worm in the center of the coil in the lower half of the picture. The cuticle has completely disappeared where the larva is exposed to host tissue but is still present, although badly damaged, in the more centrally placed regions of the coil. × 11,000.

FIG. 2. ELECTRON MICROGRAPH OF A SECTION THROUGH THE ESOPHAGUS of an infective-stage larva of *N. brasiliensis* in the skin of an immune mouse. Note the almost normal appearance of the esophagus and its cuticle. × 13,000.

FIG. 3. ELECTRON MICROGRAPH OF A SECTION THROUGH THE BODY WALL of an infective-stage larva of *N. brasiliensis* in the skin of an immune mouse. Note the disorganized appearance of the myofilaments, the collagen fibers between the muscles and the hypodermis, the absence of cuticle, and the presence of collagen fibers on the outer surface of the hypodermis. × 27,000.

FIG. 4. ELECTRON MICROGRAPH OF A SECTION THROUGH THE BODY WALL of an infective larva of *N. brasiliensis* in the skin of an immune mouse. Note the absence of the hypodermis, the slightly disorganized structure of the muscles, the almost normal appearance of the mitochondria, and the collagen fibers around the worm. × 23,000.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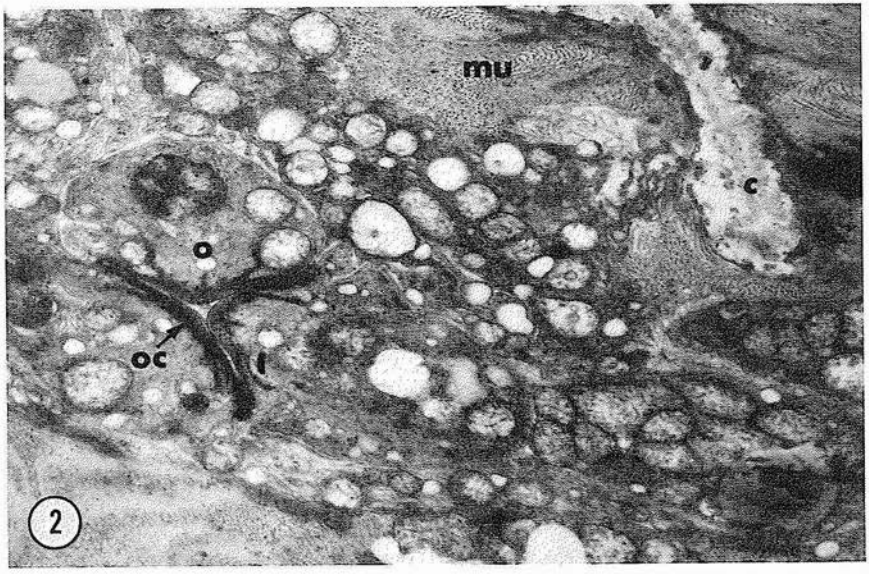
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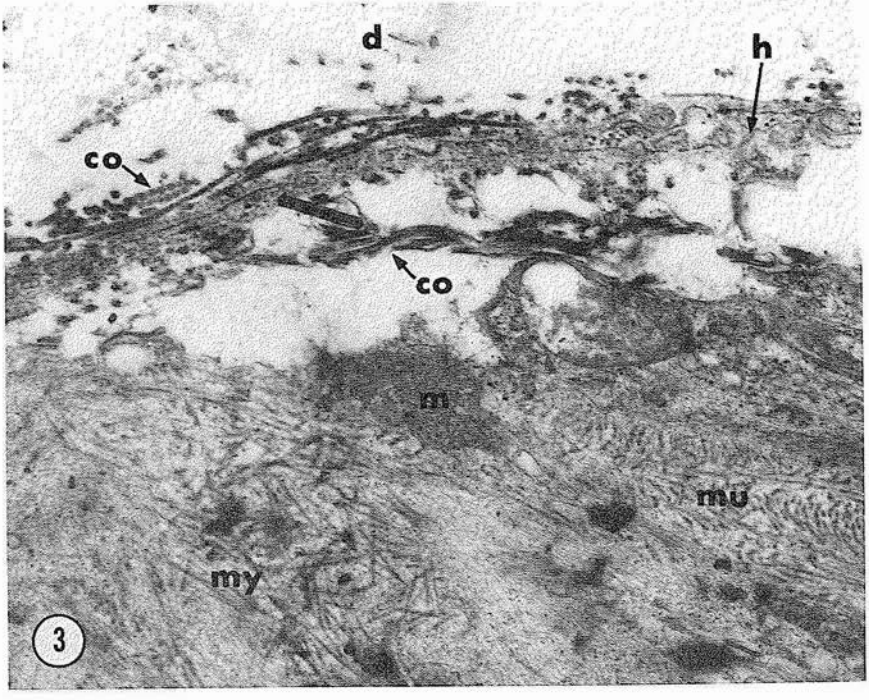
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the structure of these larvae. They were usually surrounded by bundles of collagen fibers, and host cells (such as macrophages and fibrocytes) lay among the collagen fibers. Many larvae had no cuticle, or had remnants of an obviously degenerated cuticle (figures 1-4), and fibers of collagen, apparently of host origin, were in the position normally occupied by the cuticle. The cuticle on the outside of tightly coiled worms had disappeared, and although the cuticle on the central parts of the coil was present, it had degenerated (figures 1-3). The thin hypodermis was still present in some worms, but fibers of collagen were interspersed between it and the muscles of the body wall. In others, the hypodermis had partly or entirely disappeared, with the exception of the hypodermal cords. The muscles in the more centrally placed portions of the coiled larvae were fairly normal in appearance, but the muscles towards the periphery of these worms showed varying degrees of disorganization. In the worst affected muscles the myofilaments had lost their regular arrangement and were lying in all directions within the muscle cell, although thick and thin myofilaments were still easily recognizable. Most of the mitochondria in these disorganized larval muscle cells were normal in appearance, although a few were distended. The muscle nuclei appeared normal. The esophagus, including the esophageal cuticle, and the intestine of coiled worms were apparently unaltered in structure at this time.

DISCUSSION

Recent work on the penetration of host skin by larval stages of parasitic nematodes has concentrated on the structure and behavior of these larvae as they penetrate the skin of previously uninfected animals (Lee, 1972a; Matthews, 1972). Sarles and Taliaferro (1936) showed that in rats which were actively immune to *N. brasiliensis*, the infective larvae of this same nematode were prevented from completing their normal pattern of migration within the host. In the immunized animal, larvae were killed in the skin or lungs, or were prevented from growing, establishing themselves, or laying eggs in the intestine. Taliaferro and Sarles (1937 and 1939) later showed that the larvae become coiled and immobilized in the skin within the first few hours after penetration. They suggested that this occurs because of the presence of antibody. Precipitates form in and around coiled larvae, and an intense cellular infiltration (involving host eosinophils, macrophages, lymphocytes, monocytes, and heterophils) develops in the tissues surrounding the larvae. After the worm dies it is gradually digested by the surrounding macrophages.

Ogilvie (1965) has shown that the adult worm residing in the host intestine induces greater immunity than the migrating larvae.

The work described in this paper shows that migrating larvae in the skin of immune mice are immobilized within two or three hours after application to the skin. Presumably, the immobilization is brought about by antibodies

induced by previous experience with larval and adult antigens.The larval cuticle is the first structure to be attacked by the immune host. Since the cuticle of most nematodes is a highly resistant collagenous structure, this finding is of considerable interest (see Lee, 1966 and 1972b). These results suggest that the cuticle of the immobilized larva is rapidly attacked by a collagenase that is, presumably, secreted by host defense cells surrounding the worm. The fact that the body wall cuticle covering the inner coils of the worm or located in the center of the esophagus is partially protected from degradation suggests that the observed effect is not the result of autolysis. The cytoplasmic parts of the nematode remain in a recognizable form for much longer than the cuticle, and only begin to break down once the cuticle has been destroyed. The coiled larva is quickly surrounded by collagen fibers of host origin. Following destruction of the cuticle, these fibers surround the hypodermis and eventually impose themselves between other cells of the nematode body wall. This sequence of events appears to be the beginning of the walling-off process that forms the nodule described by Taliaferro and Sarles (1937 and 1939). The speed with which the process occurs suggests that it is very efficient in protecting mice against secondary infections of *N. brasiliensis*.

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